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(54) Title: VESICLES WITH CONTROLLED RELEASE OF ACTIVES

(57) Abstract

A synthetic membrane vesicle composition containing at least one release-rate modifying agent other than a hydrohalide and at least one biologically active substance, the vesicles having defined size distribution, adjustable average size, internal chamber size and number, and a controlled release rate of the biologically active substance. A process for making the composition features addition of a release-rate modifying agent effective to prolong, sustain, and control the rate of release from the vesicles of the biologically active substance at therapeutic levels after encapsulation.

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VESICLES WITH CONTROLLED RELEASE OF ACTIVES

BACKGROUND OF THE INVENTION

1. *Field of the Invention*

The invention relates to compositions of synthetic membrane vesicles useful as a drug delivery system and to processes for their manufacture.

2. *Background of the Invention*

Multivesicular liposomes are one of the three main types of liposomes, first made by Kim, *et al.* (*Biochim, Biophys. Acta*, 782:339-348, 1983), and are uniquely different from other lipid-based drug delivery systems such as unilamellar (Huang, *Biochemistry*, 8:334-352, 1969; Kim, *et al.*, *Biochim. Biophys. Acta*, 646:1-10, 1981) and multilamellar (Bangham, *et al.*, *J. Mol. Bio.*, 13:238-252, 1965) liposomes. In contrast to unilamellar liposomes, multivesicular particles contain multiple aqueous chambers per particle. In contrast to multilamellar liposomes, the multiple aqueous chambers in multivesicular particles are non-concentric.

The prior art describes a number of techniques for producing unilamellar and multilamellar liposomes; for example, U.S. Patent No. 4,522,803 to Lenk; 4,310,506 to Baldeschwieler; 4,235,871 to Papahadjopoulos; 4,224,179 to Schneider; 4,078,052 to Papahadjopoulos; 4,394,372 to Taylor; 4,308,166 to Marchetti; 4,485,054 to Mezei; and 4,508,703 to Redziniak. The prior art also describes methods for producing multivesicular liposomes that proved unstable in biological fluids (Kim, *et al.*, *Biochim. Biophys. Acta*, 728:339-348, 1983). For a comprehensive review of various methods of unilamellar and multilamellar liposome preparation, refer to Szoka, *et al.*, *Ann. Rev. Biophys. Bioeng.*, 9:465-508, 1980.

In the method of Kim, *et al.* (*Biochim. Biophys. Acta*, 728:339-348, 1983), the encapsulation efficiency

of small molecules, such as cytosine arabinoside, was low, and had rapid release rate in biological fluids. Subsequent studies (Kim, *et al.*, *Cancer Treat. Rep.*, 71:705-711, 1987) showed that the rapid release rate of encapsulated molecules in biological fluids can be improved by encapsulating in the presence of a hydrochloride.

Optimal treatment with many drugs requires maintenance of a drug level for a prolonged period of time. For example, optimal anti-cancer treatment with cell cycle-specific antimetabolites requires maintenance of a cytotoxic drug level for a prolonged period of time. Cytarabine is a highly scheduled-dependent anti-cancer drug. Because this drug kills cells only when they are replicating DNA, a prolonged exposure at therapeutic concentration of the drug is required for optimal cell kill. Unfortunately, the half-life of Cytarabine after an intravenous (IV) or subcutaneous (SC) dose is very short, with the half-life in the range of a few hours. To achieve optimal cancer cell kill with a cell cycle phase-specific drug like Cytarabine, two major requirements need to be met: first, the cancer must be exposed to a high concentration of the drug without doing irreversible harm to the host; and second, the tumor must be exposed for a prolonged period of time so that all or most of the cancer cells have attempted to synthesize DNA in the presence of Cytarabine.

Heretofore, control of the release rate was inflexible, and the choice of release-rate modifying agents was limited primarily to hydrohalides. For a drug-delivery system, it is highly advantageous to be flexible in controlling the release rate for encapsulated substances and to have a wide choice of release-rate modifying agents.

Accordingly, it is an object of the present invention to provide a slow-releasing depot preparation

which provides a prolonged and sustained exposure of a biologically active substance at a therapeutic concentration, with a controlled release rate.

It is a further object of the present invention to provide a method of preparing such depot preparations.

Other and further objects, features, and advantages of the invention are inherent therein and appear throughout the specification and claims.

SUMMARY OF THE INVENTION

The compositions of the present invention comprise synthetic membrane vesicles, i.e. lipid vesicles with multiple internal aqueous chambers formed by non-concentric layers and wherein the chambers contain one or more release-rate modifying agents effective in slowing the release rate of the encapsulated biologically active substances. The present invention also provides methods of making such compositions.

The present synthetic membrane vesicle compositions have high encapsulation efficiency, controlled release rate of the encapsulated substance, well defined, reproducible size distribution, spherical shape, adjustable average size that can be easily increased or decreased, adjustable internal chamber size and number.

The process for producing these compositions comprises (1) mixing one or more volatile organic solvents and a lipid component containing at least one neutral lipid and at least one amphipathic lipid having one or more net negative charges; (2) adding into the organic solvent an immiscible first aqueous component containing one or more biologically active substances to be encapsulated; (3) adding to either or both the organic solvent and the first aqueous component, a release-rate modifying agent effective in slowing the release rate of the encapsulated biologically active substances; (4) forming a water-in-oil emulsion from the two immiscible components; (5) immersing the water-

in-oil emulsion into a second immiscible aqueous component; (6) dispersing the water-in-oil emulsion to form solvent spherules containing in them multiple droplets of the first aqueous component; and (7) removing the organic solvents, such as by evaporation, from the solvent spherules to form the synthetic membrane vesicles. Addition of one or more release-rate modifying agents effective in slowing the release rate of the encapsulated biologically active substances in biological fluids and *in vivo* is essential.

A BRIEF DESCRIPTION OF THE DRAWING

Figure 1 is a graph showing the rate of release of a drug from synthetic membrane vesicles suspended in human plasma at 37°C. The symbols used indicate the release rate modifying agent employed and are identified in Table 2.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The term "synthetic membrane vesicles" as used throughout the specification and claims means man-made, microscopic lipid-vesicles consisting of lipid bilayer membranes, enclosing multiple non-concentric aqueous chambers. In contrast, unilamellar liposomes have a single aqueous chamber; and multilamellar liposomes have multiple "onion-skin" type of concentric membranes, in between which are shell-like concentric aqueous compartments.

The term "solvent spherule" as used throughout the specification and claims means a microscopic spheroid droplet of organic solvent, within which is multiple smaller droplets of aqueous solution. The solvent spherules are suspended and totally immersed in a second aqueous solution.

The term "neutral lipid" means oil or fats that have no membrane-forming capability by themselves and lack a hydrophilic "head" group.

The term "amphipathic lipids" means those molecules that have a hydrophilic "head" group and hydrophobic "tail" group and have membrane-forming capability.

The term "release-rate modifying agent" means molecules other than hydrohalides added during the process of making or manufacturing the synthetic membrane vesicles that are effective in either slowing or increasing the release rate of the encapsulated biologically active substances from the synthetic membrane vesicles.

Briefly, the method of the invention comprises making a "water-in-oil" emulsion by (1) dissolving amphipathic lipids in one or more volatile organic solvents for the lipid component, (2) adding to the lipid component an immiscible first aqueous component and a biologically active substance to be encapsulated, and (3) adding to either or both the organic solvent and the first aqueous component, a release-rate modifying agent effective in slowing the release rate of the encapsulated biologically active substances from the synthetic membrane vesicles, and then emulsifying the mixture mechanically.

In the emulsion, the water droplets suspended in the organic solvent will form the internal aqueous chambers, and the monolayer of amphipathic lipids lining the aqueous chambers will become one leaflet of the bilayer membrane in the final product. The emulsion is then immersed in a second aqueous component containing one or more nonionic osmotic agents and an acid-neutralizing agent of low ionic strength, such as a proton acceptor preferably selected from free-base lysine, free-base histidine, or a combination thereof. Then the emulsion is agitated either mechanically, by ultrasonic energy, nozzle atomizations, and the like, or by combinations thereof, to form solvent spherules suspended in the second aqueous component.

The solvent spherules contain multiple aqueous droplets with the substance to be encapsulated dissolved in them. The organic solvent is removed from the spherules, preferably by evaporation of a volatile solvent, for instance by passing a stream of gas over the suspension. When the solvent is completely removed, the spherules convert into synthetic membrane vesicles. Representative gases satisfactory for use in evaporating the solvent include nitrogen, helium, argon, oxygen, hydrogen, and carbon dioxide.

The release-rate modifying agent is any molecule that is effective in slowing the rate of release of the encapsulated biologically active substances from the synthetic membrane vesicles in biological fluids and *in vivo*, with the result that the release rate of the substances is slower than that from synthetic membrane vesicles produced in the absence of such a release-rate modifying agent. The release-rate modifying agents include, but are not limited to, perchloric acid, nitric acid, formic acid, acetic acid, trifluoroacetic acid, trichloroacetic acid, sulfuric acid, phosphoric acid, and combinations thereof. The amounts of the release-rate modifying agents used is one effective to provide a prolonged, sustained, and controlled rate of release at therapeutic levels of the encapsulated biologically active substances. For example, the concentration of the release-rate modifying agent in the the organic solvent or the first aqueous component to which it is added is in the range from about 0.1 mM to about 0.5 M and preferably from about 10 mM to about 200 mM.

Many different types of volatile hydrophobic solvents such as ethers, hydrocarbons, halogenated hydrocarbons, or Freons may be used as the lipid-phase solvent. For example, diethyl ether, isopropyl and other ethers, chloroform, tetrahydrofuran, halogenated

ethers, esters and combinations thereof are satisfactory.

In order to prevent the solvent spherules from sticking to each other and to the vessel wall, it is preferred that at least 1 percent molar ratio of an amphipathic lipid with a net negative charge be included in the spherules, that the suspending second aqueous solution have a very low ionic strength, and, when an acid is used, that an agent for neutralizing the acid be added to the second aqueous solution to form a concentration of from about 0.1 mM to about 0.5 M therein to prevent coalescence of the solvent spherules to form a messy scum. In addition, one or more nonionic osmotic agents, such as trehalose, glucose, or sucrose, may optionally be used in the suspending aqueous solution to keep the osmotic pressure within and without the membrane vesicles balanced.

Various types of lipids can be used to make the synthetic membrane vesicles, and the only two requirements are that one amphipathic lipid with a net negative charge and a neutral lipid be included. Examples of neutral lipids are triolein, trioctanoin, vegetable oil such as soybean oil, lard, beef fat, tocopherol, and combinations thereof. Examples of amphipathic lipids with net negative charge are cardiolipin, the phosphatidylserines, phosphatidylglycerols, and phosphatidic acids.

The second aqueous component is an aqueous solution containing low ionic strength solutes such as carbohydrates including glucose, sucrose, lactose, and amino acids such as lysine, free-base histidine and combinations thereof.

Many and varied biological substances and therapeutic agents can be incorporated by encapsulation within the synthetic membrane vesicles.

The term "therapeutic agent" as used herein for the compositions of the invention includes, without limitation, drugs, radioisotopes, and immunomodulators. Similar substances are known or can be readily ascertained by one of skill in the art. There may be certain combinations of therapeutic agent with a given type of synthetic membrane vesicles that are more compatible than others. For example, the method for producing the synthetic membrane vesicles may not be compatible with the continued biological activity of a proteinaceous therapeutic agent. However, since conditions that would produce an incompatible pairing of a particular therapeutic agent with a particular dispersion system are well known, or easily ascertained, it is a matter of routine to avoid such potential problems.

The drugs that can be incorporated into the dispersion system as therapeutic agents include non-proteinaceous as well as proteinaceous drugs. The term "non-proteinaceous drugs" encompasses compounds that are classically referred to as drugs, such as mitomycin C, daunorubicin, vinblastine, AZT; and hormones. Of particular interest are anti-tumor cell-cycle specific drugs such as cytarabine, methotrexate, 5-fluorouracil (5-FU), floxuridine (FUDR), bleomycin, 6-mercapto-purine, 6-thioguanine, fludarabine phosphate, vincristine, and vinblastine.

Examples of proteinaceous materials that can be incorporated into the synthetic membrane vesicles are DNA, RNA, proteins of various types, protein hormones produced by recombinant DNA technology effective in humans, hematopoietic growth factors, monokines, lymphokines, tumor necrosis factor, inhibin, tumor growth factor alpha and beta, Mullerian inhibitory substance, nerve growth factor, fibroblast growth factor, platelet-derived growth factor, pituitary and hypophyseal hormones including LH and other releasing

hormones, calcitonin, proteins that serve as immunogens for vaccination, and DNA and RNA sequences.

The following TABLE 1 includes a list of representative biologically active substances effective in humans that can be encapsulated in synthetic membrane vesicles in the presence of a release-rate modifying agent of the invention, and also includes biologically active substances effective for agricultural uses.

TABLE 1

Antiasthmas	Antiarrhythmics	Tranquilizers
metaproterenol	propanolol	chlorpromazine
aminophylline	atenolol	benzodiazepine
theophylline	verapamil	butyrophenones
terbutaline		hydroxyzines
norepinephrine	Antianginas	meprobamate
ephedrine	isosorbide dinitrate	phenothiazines
isoproterenol		thioxanthenes
adrenalin		
Cardiac glycosides	**Hormones**	**Steroids**
digitalis	thyroxine	prednisone
digitoxin	corticosteroids	triamcinolone
lanatoside C	testosterone	hydrocortisone
digoxin	estrogen	dexamethasone
	progesterone	betamethasone
	mineralocorticoid	prednisolone
Antihypertensives	**Antidiabetics**	**Antihistamines**
apresoline	Diabenese	pyribenzamine
atenolol	insulin	
chlorpheniramine		
captopril		diphenhydramine
reserpine		
Antiparasitics	**Antineoplastics**	**Sedatives and Analgesics**
praziquantel	azathioprine	morphine
metronidazole	bleomycin	dilaudid
pentamidine	cyclophosphamide	codeine
ivermectin	vincristine	codeine-like
synthetics		
	methotrexate	demerol
Nucleic Acids and Analogs	6-TG	oxymorphone
DNA	6-MP	phenobarbital
RNA	vinblastine	barbiturates

methylphosphonates and analogs
Antisense nucleic acids

VP-16
VM-26
cisplatin
5-FU
FUDR
fludarabine phosphate

fentanyl
ketorolac

Antibiotics
penicillin
tetracycline
amikacin
erythromycin
cephalothin
imipenem
cefotaxime
carbenicillin
ceftazidime
kanamycin
tobramycin
ampicillin
gentamycin
cefoxitin
cefadroxil
cefazolin
other aminoglycosides
amoxicillin
moxalactam
piperacillin
vancomycin
ciprofloxacin
other quinolones

Immunodulators
interferon
interleukin-2
gammaglobulin
monoclonal antibodies

Vasopressors
dopamine
dextroamphetamine

Antifungals
amphotericin B
myconazole
muramyl dipeptide
clotrimazole
ketoconozole
fluconazole
itraconazole

Antivirals
acyclovir and derivatives
Gancyclovir and phosphates
Winthrop-51711
ribavirin
rimantadine/amantadine
azidothymidine & derivates
adenine arabinoside
amidine-type protease inhibitors

Vaccines
other recombinant, killed and live vaccines and antigenic material for use as vaccines.
antigenic material for the treatment of allergies
influenza
respiratory syncytial virus
HIV vaccine
Hemophilus influenza vaccines
Hepatitis A,B,C vaccines
mumps
rubella
measles
tetanus
malaria vaccines
herpes
cancer vaccines
Anti-leu-3a vaccine

Monoclonal Antibodies (human, mouse other species-derived and/or recombinant and/ or fusions and/or fragments thereof)
OKT3
OKT4
HA-1A
Anti-Carcino-Embryonic Antigen Antibodies

Anti-Ganglioside Antibodies: Anti GD2, Anti GM2, Anti GD3, Anti GM3
Urinary Tract-Associated Antigen-related antibodies
Anti-Il-2 Receptor
Chimeric Anti-Leu-2
Anti-IL-2 receptor
Anti-Leu-2
Chimeric Anti-Leu-3a
Chimeric L6
MAb-L6
Radiolabeled L6
Centorex
Centoxin
Panorex
Anti-LPS
Immunotoxin
Anti-tumor necrosis factor
Anti-pseudomonas
Anti-tumor necrosis factor
OncoRad 103
OncoScint CR103
OncoScint OV103
OncoScint PR356
OncoTher 130
KS 1/4-DAVLB
ADCC agent
Murine monoclonal antibodies to human B-cell lymphomas (anti-idiotypes)
Murine monoclonal antibody (lMelpgl) (anti-idiotype) against murine monoclonal antibody to melanoma-associated antigen
Anti-B4-blocked ricin
Anti-My9-blocked ricin
ImmuRaid-CEA
MAb against colorectal, ovarian, and lung cancers
rhenium-186 MAb
Orthoclone OKT®
E5™
LYM-1
TNT
XomaZyme®-791
XomaZyme®-CD5 Plus
XomaZyme®-CD7 Plus
XomaZyme®-Mel

<u>Herbicides</u>
Triazine
chloroacetamide
cyanazine
bentazone
Roundup
Rodeo
butachlor
CNP
chlomethoxynil
simetryne
Atrazine
Alachlor

Cyanazine
metolachlor
metribuzin
phenoxy herbicides: 2,4-D [(2,4-dichlorophenoxy)acetic acid],
2,4-D amine (2,4-dichlorophenoxyacetic acid dimethylamine),
2,4-D isooctyl (2,4-dichlorophenoxyacetic acid isooctyl ester),
2,4,5-T amine (2,4,5-trichlorophenoxyacetic acid trimethylamine)
other triazine herbicides
other chloroacetamide herbicides
other phenoxyacid herbicides

Pesticides
Abamectin
other avermectins
atrazine
lindane
dichlorvos
dimethoate
warfarin
p,p'-DDD
p,p'-DDE
HCH
DMDT
aldrin
dieldrin
Aldicarb
EDB
DCP
DBCP
simazine
cyanazine
Bacillus thuringiensis toxin
Bacillus thuringiensis var. kurstaki
bis(tri-n-butyltin)oxide (TBTO)
other organochlorine pesticides

Proteins and Glycoproteins
lymphokines
interleukins - 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11.
cytokines
GM-CSF
M-CSF
G-CSF
tumor necrosis factor
inhibin
tumor growth factor
Mullerian inhibitors substance
nerve growth factor
fibroblast growth factor
platelet derived growth factor
coagulation factors (e.g. VIII, IX, VII)
insulin
tissue plasminogen activator
histocompatibility antigen
oncogene products
myelin basic protein

collagen
fibronectin
laminin
other proteins made by recombinant DNA technology
erythropoietin
IL-3/GM-CSF fusion proteins
Monoclonal antibodies
Polyclonal antibodies
antibody-toxin fusion proteins
antibody radionuclide conjugate
Interferons
Fragments and peptide analogs, and analogs of fragment of proteins, peptides and glycoproteins.
Epidermal growth factor
CD4 receptor and other recombinant receptors
other proteins isolated from nature
Antidiuretic hormone
oxytocin
adrenocorticotropin Hormone
calcitonin
follicle stimulating hormone
luteinizing hormone releasing hormone
luteinizing hormone
gonadotrophin
transforming growth factors
streptokinase
Human Growth Hormone,
Somatotropins for other species, including, but not limited to:

1. Porcine,
2. Bovine,
3. Chicken,
4. Sheep,
5. Fish,

Growth Hormone releasing hormones for humans and various animal species,
Glucagon,
Desmopressin,
Thyroid Releasing Hormone,
Thyroid Hormone,
Secretin,
Magainins,
Integrins,
Adhesion Peptides, including, but not limited to, those having the Arginine-Glutamine-Aspartic Acid sequence,
Super Oxide Dismutase,
Defensins,
T-Cell Receptors,
Bradykinin antagonists,
Pentigetide,
Peptide T,
Antinflammins,
Major Histocompatibility (MHC) complex components and peptides targeted to the MHC,
Protease inhibitors,

Lypressin,
Buserelin,
Leuprolide,
Nafarelin,
Deslorelin,
Goserelin,
Historelin,
Triptorelin,
LHRH antagonists,
HOE-2013,
Detirelix,
Org-30850,
ORF-21243,
Angiotensin Converting Enzyme inhibitor Peptide,
Renin inhibitory peptides,
Ebiratide (HOE-427),
DGAVP,
Opiate receptor agonists and antagonists, including, but not limited to:

1. Enkephalins,
2. Endorphins,

E-2078,
DPDPE,
Vasoactive intestinal peptide,
Atrial Natriuretic Peptide,
Brain Natriuretic Peptide,
Atrial Peptide clearance inhibitors,
Hirudin,
Oncogene Inhibitors,
Other Colony Stimulating Factors,

Neurotransmitters	Radionuclides	Radio contrasts
Dopamine	Technetium	Gadolinium chelates
Epinephrine	Indium	Iohexol
Norepinephrine	Yttrium	Ethiodol
acetylcholine	Gallium	Iodexinol
Gammaamino butyric acid		

Others
cell surface receptor blockers

The term "therapeutically effective" as it pertains to the compositions of the invention means that a therapeutic agent is present in the aqueous phase within the vesicles at a concentration sufficient to achieve a particular medical effect for which the therapeutic agent is intended. Examples, without limitation, of desirable medical effects that can be attained are chemotherapy, antibiotic therapy, and regulation of metabolism. Exact dosages will vary

depending upon such factors as the particular therapeutic agent and desirable medical effect, as well as patient factors such as age, sex, general condition, and the like. Those of skill in the art can readily take these factors into account and use them to establish effective therapeutic concentrations without resort to undue experimentation.

Generally, however, the dosage range appropriate for human use includes the range of 0.1-6000 mg/sq m of body surface area. For some applications, such as subcutaneous administration, the dose required may be quite small, but for other applications, such as intraperitoneal administration, the dose desired to be used may be very large. While doses outside the foregoing dose range may be given, this range encompasses the breadth of use for practically all the biologically active substances.

The synthetic membrane vesicles may be administered for therapeutic applications by any desired route; for example, intramuscular, intrathecal, intraperitoneal, subcutaneous, intravenous, intralymphatic, oral and submucosal, under many different kinds of epithelia including the bronchialar epithelia, the gastrointestinal epithelia, the urogenital epithelia, and various mucous membranes of the body.

In addition the synthetic membrane vesicles of the invention can be used to encapsulate compounds useful in agricultural applications, such as fertilizers, pesticides, and the like. For use in agriculture, the synthetic membrane vesicles can be sprayed or spread onto an area of soil where plants will grow and the agriculturally effective compound contained in the vesicles will be released by contact with rain and irrigation waters. Alternatively the slow-releasing vesicles can be mixed into irrigation waters to be applied to plants and crops. One skilled in the art will be able to select an effective amount of the

compound useful in agricultural applications to accomplish the particular goal desired, such as the killing of pests, the nurture of plants, etc.

The synthetic membrane vesicles may be modified in order to impart organ or cell target specificity, for instance by incorporating them into a targeted delivery system. Such modifications may be particularly relevant for using the synthetic membrane vesicles of the invention to administer drugs that are highly toxic or capable of inducing severe side effects, such as taxol.

The targeting of the synthetic membrane vesicles is classified based on anatomical and mechanistic factors. In anatomical targeting, the synthetic membrane vesicle is targeted to a specific body location, for example, organ-specific, cell-specific, and organelle-specific targeting. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of the synthetic membrane vesicles of the invention to distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries. In active targeting, on the other hand, the synthetic membrane vesicle is incorporated into a targeted delivery system by coupling it to a specific ligand, such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the synthetic membrane vesicles in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization (see, for example, *Remington's Pharmaceutical Sciences*, Gannaro, A.R., ed., Mack Publishing, 18 Edition, pp. 1691-1693, 1990)

In general, the compounds to be bound to the surface of the synthetic membrane vesicles will be ligands and receptors that allow the dispersion system

to actively "home in" on the desired tissue. A ligand may be any compound of interest that will specifically bind to another compound, referred to as a receptor, such that the ligand and receptor form a homologous pair.

The surface of the targeted delivery system can be modified in a variety of ways. For instance, lipid groups can be incorporated into the lipid bilayer of the synthetic membrane vesicles in order to maintain the targeting ligand in stable association with the lipid bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand (Mannino, *et al.*, *Bio Techniques*, 6(7):682, 1988). The compounds bound to the surface of the synthetic membrane vesicles may vary from small haptens of from about 125-200 molecular weight to much larger antigens with molecular weights of at least about 6000, but generally of less than 1 million molecular weight. Proteinaceous ligand and receptors are of particular interest.

In general, the surface membrane proteins that bind to specific effector molecules are referred to as receptors. In the present invention, the preferred receptors are antibodies. These antibodies may be monoclonal or polyclonal and may be fragments thereof such as Fab $F(ab')_2$, and F_v, which are capable of binding to an epitopic determinant. Techniques for binding of proteins, such as antibodies, to synthetic membrane vesicles are well known (see, for example, U.S. 4,806,466 and U.S. 4,857,735, incorporated by reference).

Antibodies can be used to target the synthetic membrane vesicles to specific cell-surface ligands. For example, certain antigens expressed specifically on tumor cells, referred to as tumor-associated antigens (TAAs) may be exploited for the purpose of targeting antibody-containing synthetic membrane vesicles

directly to malignant tumors. Since the composition incorporated into the synthetic membrane vesicles may be indiscriminate with respect to cell type in its action, a targeted synthetic membrane vesicles offers a significant improvement over randomly injecting non-specific synthetic membrane vesicles. A number of procedures can be used to covalently attach either polyclonal or monoclonal antibodies to a bilayer of the synthetic membrane vesicles. Antibody-targeted synthetic membrane vesicles can include monoclonal or polyclonal antibodies or fragments thereof such as Fab, or $F(ab')_2$, as long as they bind efficiently to the antigenic epitope on the target cells. Synthetic membrane vesicles may also be targeted to cells expressing receptors for hormones or other serum factors (Malone, *et al.*, *Proc. Nat'l. Acad. Sci, USA*, 86:6077, 1989; Gregoriadis, *Immunology Today*, 11(3):89, 1990; both incorporated by reference).

The following examples illustrate the manner in which the invention can be practiced. It is understood, however, that the examples are for the purpose of illustration and the invention is not to be regarded as limited to any of the specific materials or conditions therein.

EXAMPLE 1

Step 1) In a clean glass cylinder (2.5 cm inner diameter X 10.0 cm height), 5 ml of a solution containing 46.5 μmoles of dioleoyl phosphatidylcholine, 10.5 μmoles of dipalmitoyl phosphatidylglycerol, 75 μmoles of cholesterol, 9.0 μmoles of triolein in chloroform were placed (the lipid phase).

Step 2) Five ml of aqueous phase, cytarabine (20 mg/ml) dissolved in 0.136 N perchloric acid, a release-rate modifying agent, is added into the above glass cylinder containing lipid phase. The osmolarity of the aqueous solution is about 274 ± 20 mOs/kg. For the

other release-rate modifying agents namely, nitric acid, formic acid, sulfuric acid, phosphoric acid, acetic acid, trichloroacetic acid, and trifluoroacetic acid, 20 mg/ml solutions of cytarabine were prepared with these agents to yield aqueous solutions that are nearly isotonic with respect to the final storage medium, namely normal saline (0.9% sodium chloride).

Step 3) For making the water-in-oil emulsion, a homogenizer (AutoHomoMixer, Model M, Tokushu Kika, Osaka, Japan) was used by mixing for 8 minutes at a speed of 9000 rpm.

Step 4) For making the chloroform spherules suspended in water, 20 ml of a solution containing 4 percent dextrose and 40 mM lysine was layered on top of the water-in-oil emulsion, and then mixed for 60 seconds at a speed of 4000 rpm to form the chloroform spherules.

Step 5) The chloroform spherule suspension in the glass cylinder was poured into the bottom of a 1000 ml Erlenmeyer flask containing 30 ml of water, glucose (3.5 g/100 ml), and free-base lysine (40 mM). A stream of nitrogen gas at 7 l/minute was flushed through the flask to slowly evaporate chloroform over 20 minutes at 37°C. 60 ml of normal saline (0.9% sodium chloride) was added to the flask. The synthetic membrane vesicles were then isolated by centrifugation at 600 X g for 10 minutes. The supernatant was decanted, and the pellet was resuspended in 50 ml of normal saline. The pellet was resuspended in saline to yield a final concentration of 10 mg Cytarabine per ml of suspension.

The average length-weighted mean diameter of the resulting synthetic membrane vesicles particles is in the range from 12-16 μm. Percentage of capture of Cytarabine is given in TABLE 2. The use of different release-modifying agents had marked influence on the rate of Cytarabine release from the synthetic membrane vesicles incubated in human plasma. The percent of

Cytarabine retained in the synthetic membrane vesicles after incubation at 37°C in human plasma for the different acids is plotted as a function of time of incubation in Figure 1. The half-life of drug release, calculated assuming a single-exponential model for the data shown in Figure 1, is given in TABLE 2. The data in TABLE 2 are the mean and standard deviation from three experiments.

TABLE 2

Acid	Percent Capture of Cytarabine	Half-Life in Days for Release of Cytarabine	Symbol in Figure 1
Hydrochloric Acid	49 ± 5	65.7 ± 4.4	◆
Perchloric Acid	45 ± 5	37.2 ± 8.0	▼
Nitric Acid	44 ± 3	54.5 ± 5.7	■
Phosphoric Acid	72 ± 1	6.5 ± 0.2	▲
No Acid	46 ± 2	5.3 ± 0.5	●
Formic Acid	37 ± 2	5.6 ± 0.2	◇
Trichloroacetic Acid	29 ± 1	5.5 ± 0.6	▽
VAcetic Acid	30 ± 2	4.8 ± 0.5	□
Trifluoroacetic Acid	35 ± 1	3.4 ± 0.4	△
Sulfuric Acid	57 ± 4	1.6 ± 0.5	○

It was surprising and unexpected that the nature of the acid had a profound effect on the release rates of cytarabine in human plasma. Use of monoprotic inorganic acids, namely, hydrochloric acid, nitric acid, and perchloric acid, resulted in the slowest release rate for cytarabine. Diprotic and triprotic acids, i.e., sulfuric acid and phosphoric acid, resulted in fast release rates. The organic acids, formic acid, acetic acid, trifluoroacetic acid and trichloroacetic acid, also resulted in fast release rates.

Thus, the present disclosure provides "depot" preparations of wide application and uses in which biologically active substances are encapsulated in

biologically active substances are encapsulated in relatively large amounts, provide prolonged exposure or delivery at therapeutic concentrations of these substances for optimal results, and the release rate of the substance is controlled by varying the nature of the acid used in the formulation.

The present invention, therefore, is well suited and adapted to attain the ends and objects and has the advantages and features mentioned as well as others inherent therein.

While presently preferred embodiments of the invention have been given for the purpose of disclosure, changes may be made therein which are within the spirit of the invention as defined by the scope of the appended claims.

CLAIMS

1. A composition comprising a synthetic membrane vesicle comprising lipid bilayer membranes enclosing multiple non-concentric aqueous chambers containing one or more biologically active substance encapsulated therein and one or more non-hydrohalide release-rate modifying agents.
2. The composition of claim 1, wherein the release-rate modifying agents are selected from the group consisting of nitric acid, perchloric acid, formic acid, sulfuric acid, phosphoric acid, acetic acid, trichloroacetic acid, and trifluoroacetic acid, and salts or combinations thereof.
3. The composition of claim 1 wherein the release-rate modifying agent is a monoprotic inorganic acid.
4. The composition of claim 2, wherein the acids are neutralized with a proton acceptor.
5. The composition of claim 1 wherein the biologically active substance is a drug. substance.
6. The composition of claim 1 wherein the biologically active substances are selected from the group consisting of antibiotics, vaccines, antivirals, antifungals, anti-tumor drugs, proteins and glycoproteins.
7. A composition of claim 6 wherein the anti-tumor drug is cytarabine.
8. A composition of claim 1 wherein the biologically active substances are selected from the group consisting of herbicides and pesticides.
9. A targeted delivery system comprising a composition of Claim 1 with a targeting ligand attached thereto.
10. A targeted delivery system of claim 9 wherein the targeting ligand is an antibody or fragment thereof.

11. A targeted delivery system of claim 9 wherein the antibody is a monoclonal antibody.

12. A targeted delivery system of claim 9 wherein lipid groups are incorporated into the lipid bilayer of the synthetic membrane vesicle.

13. The composition of claim 1 wherein the synthetic membrane vesicle is anatomically targeted.

14. The composition of claim 1 wherein the synthetic membrane vesicle is mechanistically targeted.

15. The composition of claim 1 wherein the synthetic membrane vesicle is passively targeted.

16. The composition of claim 1 wherein the synthetic membrane vesicle is actively targeted.

17. The composition of claim 16 wherein the synthetic membrane vesicle is actively targeted by coupling with a moiety selected from the group consisting of a sugar, a glycolipid, and a protein.

18. A synthetic membrane vesicle of claim 1 produced by the method comprising:
    (a) forming a water-in-oil emulsion from two immiscible components containing at least one organic solvent, water, at least one biologically active substance, and at least one non-hydrohalide release-rate modifying agent;
    (b) dispersing the said water-in-oil emulsion into an aqueous component to form solvent spherules; and
    (c) removing the organic solvent from the solvent spherules to form the synthetic membrane vesicle.

19. A process for producing synthetic membrane vesicles comprising the steps of:
    (a) forming a water-in-oil emulsion from two immiscible components containing at least one organic solvent, water, at least one biologically active substance, and at least one non-hydrohalide release-rate modifying agent;
    (b) dispersing the water-in-oil emulsion into an aqueous component to form solvent spherules; and
    (c) removing the organic solvent from the solvent spherules to form the synthetic membrane vesicles containing aqueous droplets with the biologically active substance and the release rate modifying agent dissolved therein..

20. The process of claim 19 wherein, the concentration of the non-hydrohalide release-rate modifying agent is present in the range of about 0.1 mM to about 0.5 M.

21. The process of claim 19 wherein, an acid neutralizing agent in a concentration of from about 0.1 mM to about 0.5 M is added during step (b).

22. The process of claim 19 where, the organic solvent has a dissolved lipid component containing at least one amphipathic lipid with a net negative charge and at least one neutral lipid.

23. The process according to claim 22 wherein the lipid component is selected from the group consisting of a phospholipid and an admixture of phospholipids.

24. The process according to claim 23 wherein, the phospholipids are selected from the group consisting of phosphatidylcholine, cardiolipin, phosphatidylethanolamine, sphingomyelin, lysophosphatidylcholine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, and phosphatidic acid.

25. The process according to claim 24 wherein, at least one of the phospholipids has at least one net negative charge.

26. The process according to claim 24 wherein, the phospholipid is provided in admixture with cholesterol.

27. The process according to claim 24 wherein, the phospholipid is provided in admixture with stearylamine.

28. The process according to claim 22 wherein, a lipophilic biologically active material is provided in admixture with the lipid component.

29. The process according to claim 22 wherein, the neutral lipid is selected from the group consisting of triolein, trioctanoin, vegetable oil, lard, beef fat, tocopherol, and combinations thereof.

30. The process according to claim 19 wherein, the organic solvent is selected from the group consisting of ethers, hydrocarbons, halogenated hydrocarbons, halogenated ethers, esters, and combinations thereof.

31. The process according to claim 19 wherein, the biologically active material is hydrophilic.

32. The process according to claim 19 wherein, the emulsion is formed using a method selected from the group consisting of mechanical agitation, ultrasonic energy, and nozzle atomization.

33. The process according to claim 32 wherein, the average size of the synthetic membrane vesicles and number of the aqueous chambers therewithin are determined by the type, intensity, and duration of the emulsification method selected.

34. The process according to claim 19 wherein, the release rate modifying agent is a monoprotic inorganic acid, and aqueous component contains at least one neutralizing agent.

35. The process according to claim 34 wherein, the neutralizing agent is selected from the group consisting of free-base lysine, free base histidine, and a combination thereof.

36. The process according to claim 34 wherein, the aqueous component is an aqueous solution containing solutes selected from the group consisting of carbohydrates and amino acids.

37. The process according to claim 34 wherein, the aqueous component is an aqueous solution containing solutes selected from the group consisting of glucose, sucrose, lactose, free-base lysine, free-base histidine, and combinations thereof.

38. The process according to claim 19 wherein, the solvent spherules are formed using a method selected from the group consisting of mechanical agitation, ultrasonic energy, nozzle atomization, and combinations thereof.

39. The process according to claim 38 wherein, the average size of the synthetic membrane vesicle is determined by the type, intensity, and duration of the energy used.

40. The process according to claim 19 wherein, the organic solvent is removed by passing gas over the aqueous component.

41. The process of Claim 19 wherein, the biologically active substance is selected from the group consisting of antiasthmas, cardiac glycosides, antihypertensives, antiparasitics, nucleic acids and analogs, antibiotics, vaccines, antiarrhythmics, antianginas, hormones, antidiabetics, antineoplastics, immunomodulators, antifungals, tranquilizers, steroids, sedatives and analgesics, vasopressors, antivirals, monoclonal antibodies, herbicides, pesticides, proteins and glycoproteins, neurotransmitters, radionuclides, radio contrasts, and combinations thereof.

42. The synthetic membrane vesicle of claim 32 or 33 wherein, the biologically active substance is selected from the group consisting of antiasthmas, cardiac glycosides, antihypertensives, antiparasitics, nucleic acids and analogs, antibiotics, vaccines, antiarrhythmics, antianginas, hormones, antidiabetics, antineoplastics, immunomodulators, antifungals, tranquilizers, steroids, sedatives and analgesics, vasopressors, antivirals, monoclonal antibodies, herbicides, pesticides, proteins and glycoproteins, neurotransmitters, radionuclides, radio contrasts, and combinations thereof.

43. A method for the treatment of a patient with a biologically active compound comprising:
administering a therapeutic amount of a therapeutic agent to a patient encapsulated in a synthetic membrane vesicle in the presence of a non-hydrohalide release-rate modifying agent effective to control the rate of release of the compound at a therapeutic level.

44. A method for the treatment of a patient with a biologically active compound comprising:
administering to the patient a synthetic membrane vesicle of claims 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

45. The method of claim 19 wherein, the biologically active substance is selected from the group consisting of herbicides and pesticides.

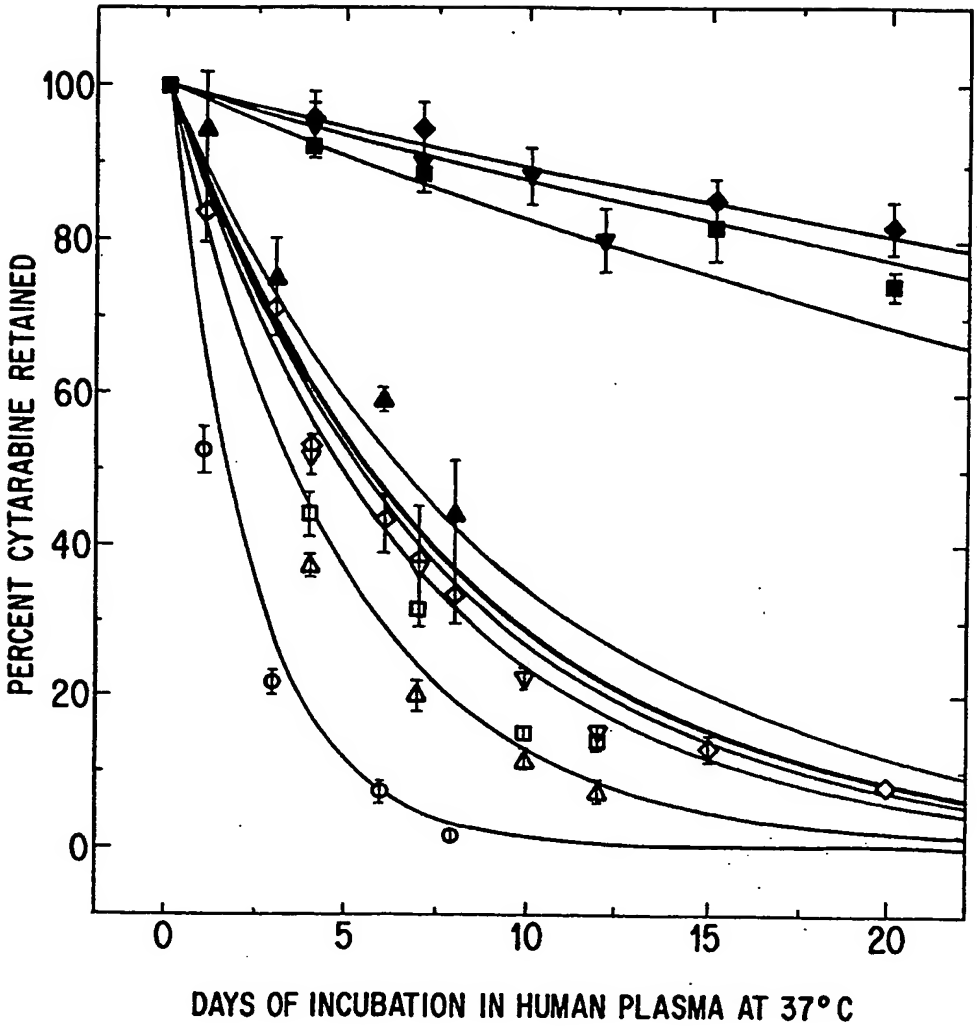
PERCENT CYTARABINE RETAINED
100
80
60
40
20
0
0
5
10
15
20
DAYS OF INCUBATION IN HUMAN PLASMA AT 37° C

FIG. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/12957

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 9/127
US CL :424/450
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/450, 417

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CANCER RESEARCH 53, April 1, 1993 (KIM) "Prolongation of Drug Exposure in Cerebrospinal Fluid by Encapsulation into DepoFoam" pp. 1596-1598, see entire document.	1, 3-7, 9-10, 12-19, 21-26, 28, 30-44
Y	US, A, 4,310,506 (BALDESCHWIELER) 12 January 1982, see columns 3 and 4 and Examples II and III.	1, 19, 27
Y	US, A, 4,224,179 (SCHNEIDER) 23 September 1980, see columns 7-9.	1, 19, 27, 29
Y	US, A, 4,752,425 (MARTIN) 21 June 1988, see entire document.	19, 32
Y	US, A, 4,920,016 (ALLEN) 24 April 1990, see columns 4-22.	1, 11, 16, 17

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be part of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/12957

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,211,955 (LEGROS) 18 May 1993, see entire document.	1, 2, 19, 20
Y	US, A, 4,588,578 (FOUNTAIN) 13 May 1986, see columns 6, lines 8-19 and column 13, lines 60-68.	8, 45